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Coprinellus mitrinodulisporus, a new species from chamois dung

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Abstract — The genus *Coprinellus* is re-examined from its establishment, through demotion as a synonym of *Coprinus,* and up through its current reinstatement. An agaric with a setulose pileus, sphaerocystic veil, and mitriform, nodulose spores has been isolated from chamois dung and, based on morphological data, is regarded as a new species in *Coprinellus*. The new taxon is compared with morphologically similar *Coprinellus* species, particularly with those having mitriform spores. Other taxa recently described in *Coprinus* are transferred to *Coprinellus*.

Key words — *Agaricales*, *Setulosi*, 28S rDNA, ITS, β-tubulin

Introduction

Persoon (1797) erected the genus *Coprinus* to accommodate agaric species with an ephemeral, membranous cap and blackening, deliquescent gills. Karsten (1879) later established the genus *Coprinellus* for species differing from *Coprinus* in having "caps covered by a cuticle or veil, finally lacerate and turned upwards" rather than "scaly from remnants of the universal veil, and covered by a veil". Ricken (1915), who accepted *Coprinellus* as a subgenus of *Coprinus* limited to non-deliquescent species, restricted subgen. *Coprinus* to species with deliquescent gills. Lange (1938) reinstated *Coprinellus* at the genus level to include some non-deliquescent species, which Singer (1986) later placed in *Coprinus* subsect. *Setulosi* J.E. Lange. Singer (1986), whose conceptions were basic to the modern taxonomy of *Agaricales* Underw., regarded *Coprinellus* as a later synonym of *Coprinus*.

M. Lange (1952), who studied pileocystidiate species from different geographical origins morphologically and with interfertility tests, showed that

some species consisted of more than one cryptic, intersterile entity. Uljé & Bas (1991) monographed the *setulosi* at subsection level of section *Pseudocoprinus* (Kühner) P.D. Orton & Watling and included species with a hymenidermal cuticle and setulae on cap and stem, sometimes in association with veil remnants.

Molecular phylogenetic studies (Hopple & Vilgalys 1994, 1999; Johnson & Vilgalys 1998; Johnson 1999; Moncalvo et al. 2000, 2002) show *Coprinus comatus* (O.F. Müll.) Pers. (the type species of *Coprinus*) and its allies as distantly related to the other *Coprinus* species, and reveal *Coprinus* sensu lato to be a heterogeneous, polyphyletic assemblage. Based on these results, Redhead et al. (2001) split *Coprinus* s.l. into four genera — *Coprinus* s. str. in *Agaricaceae* Chevall. and *Coprinellus*, *Coprinopsis* P. Karst., and *Parasola* Redhead et al. in *Psathyrellaceae* Vilgalys et al. Their concept of *Coprinellus* includes species that in traditional systematics belong to subsect. *Setulosi* and subsects. *Domestici* Singer and *Micacei* (Fr.) Uljé & Noordel. of sect. *Veliformes* (Fr.) Penn. and are characterised by a hymenidermal or cystodermal pileipellis with a globular veil and/or pileocystidia (setulae).

This new taxonomy based on phylogenetic relationships in association with morphological features is now accepted by many authors, including Keirle et al. (2004) in their research on Hawaiian *Agaricales*, Nagy et al. (2009), who applied it to a complex study on *Parasola,* and Schafer (2010), who combined earlier subsections as sections of *Parasola*, *Coprinellus,* and *Coprinopsis*.

Since Uljé & Bas (1991) monographed *Setulosi*, additional new *Coprinus* s.l. species belonging to this section have been published (Uljé & Verbeken 2002, Uljé & Keizer 2003, Uljé & Noordeloos 2003, Nagy 2006), a few of which have been recombined in *Coprinellus* (Nagy et al., in press).

Our systematic study of coprophilous ascomycetes and basidiomycetes from Italy has recently allowed us to observe the growth on dung, in a damp chamber culture, of a *Coprinus* s.l., whose morphological features match those of *Setulosi*, but whose combination of characters does not correspond to any species in this section. We describe it here as a new species of *Coprinellus*.

Materials and methods

Isolation of the fungus — Morphological studies

Samples of chamois (*Rupicapra rupicapra*) dung were dried and cultured, after nineteen months, in a non-sterilised damp chamber according to Richardson & Watling (1997) and Richardson (2001), slightly modified by Doveri (2004). The cultures, placed under natural light at room temperature (18–25°C), were observed daily for five weeks with the unaided eye and a ×7–45 magnification stereomicroscope. The macroscopic features were immediately described, and fresh material was mounted in water and Congo red and microscopically examined under a binocular light microscope. Spore

size was measured in water and calculated on 80 mature spores from 3 basidiomata, excluding the apiculum from the measurements (Q means the quotient of length divided by the breadth in face view). Small fruitbodies were dried in a few minutes with an artificial light. The collection has been preserved as dried material and slides (PI). Herbarium abbreviation follows Holmgren & Holmgren (1998).

Molecular studies

DNA extraction was performed on a dried fruitbody using the DNeasy Plant MiniKit (Qiagen), according to the manufacturer's protocol. Polymerase Chain Reaction (PCR) was used to amplify the LSU and the ITS regions of the nuclear ribosomal DNA, employing the following primers: LR7, LR5, LR3R and LROR for the first 1.5 kb of the LSU gene and ITS1 and ITS4 for the ITS region (Gardes & Bruns 1993). Amplification reaction mixtures contained 25–50 ng of template DNA, GoTaq®Green Master Mix (Promega) 1X and 0.5 mM of each primer in a volume of 50 µL.

Amplification was performed in a GeneAmp® PCR System 2400 (Perkin Elmer) using the following parameters: for LSU initial denaturation step at 94°C for 5 min, 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 50°C (for LROR/LR7) or 52°C (for LROR/LR5 and LR3R/LR7) for 1 min and extension at 72°C for 2 min, final extension of 72°C for 7 min; for ITS initial denaturation step at 94°C for 1 min, 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 54°C for 1 min and extension at 72°C for 1 min, final extension of 72°C for 4 min. After the final extension of 72°C reactions were held at 4°C.

In addition, a fragment of the β-tubulin gene was amplified by primers B36f_psa/B12r_psa according to Nagy et al. (2010).

PCR products were purified by the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's protocol and submitted to sequencing. Samples to be sequenced were processed by the DNA Sequence Facility at the Bio Molecular Research (BMR), Servizio di Sequenziamento – CRIBI, University of Padova (Italy). For sequencing the same primers as described above for the ITS fragments and LR16 or LR22 as additional primers for the LSU fragment (Gardes & Bruns 1993) were used. The LSU and ITS sequences derived from these studies have been deposited in GenBank and compared with other sequences in GenBank.

Taxonomy

Coprinellus mitrinodulisporus Doveri & Sarrocco **sp. nov.**

MycoBank 518735; GenBank HQ180170 Plate 1-2

Pileus primo subglobosus vel elliptico-paraboliformis, usque ad 2 mm altus, deinde convexo-conicus vel campanulato-convexus, ultimo convexo-applanatus vel etiam revolutus, 3–10 mm latus, dense pruinoso-pubescens, radialiter striatus, deinde fissuratus. Cuticula primo ochracea, luteo vel purpureo, raro olivaceo, soffusa, deinde floris lactis colorem accipiens, plerumque ad marginem pallidior, ad postremum cinerascens. Lamellae ascendentes, stipitem non attinentes, ventricosae, infrequentes, ex albo nigricantes, ad marginem pallidiores. Stipes usque ad 45 × 0.5–0.8 mm, albidus vel modice quam pileus pallidior, flexuosus, cylindratus, aliquanto ad basim dilatatus at non bulbosus, omnino pruinoso-pubescens, saepe basali radiato mycelio praeditus. Inodorus. Velum granulatum, et ad

pileum et ad stipitem conspersum, ex crasse crustatis atque crassitunicatis sphaerocytibus, 12–20 µm diam., compositum. Sporae (9–) 9.5–11 (–11.5) × 6–7 x 5–6 µm, in adverso visu mitriformes, a latere subamygdaliformes, plerumque rotunde quadrinodosae, fuscobadiae, valde excentrico, 1.5–2 µm lato, poro germinativo praeditae. Basidia 16–27 × 6–9 µm, tetraspora, claviformia vel subcylindrata. Pleurocystidia absentia. Cheilocystidia copiosa, globosa vel late ellipsoidea, pedicularia, 25–39 × 21–32 µm. Pileipellis ex globosis, claviformibus vel late ellipsoideis, interdum crustatis cellulis, 20–48 × 16–34 µm, composita. Pileocystidia copiosa, lageniformia, et tenuitunicata, 62–78 (–90) × 12–15 µm, ad acutum apicem contracta, aliquando crustata leptocystidia, et crassitunicata, 35–45 × 11–16 µm, plerumque crustata sclerocystidia. Caulocystidia copiosa, pileocystidiis similia, 40–75 × 10–17 µm. Fibulae absentes. Holotypus hic designatus N.A. 1 in Pisani Horti Botanici viridario conservatur, ex fimo Rupicaprae rupicaprae, in Augustana Italica terra (saltus Salati) invento atque culto, ad viginti solitaria specimina remota, 28 Augustus 2008.

Type: Salati pass (45°52'34"N 7°52'05"E), Aosta, Italy, on chamois dung, 28.8.2008, leg.: L. Levorato (**Holotype** N.A. 1, Pisa Botanical Garden)

Etymology: *mitri-noduli-sporus* from the Latin (in turn from the Greek) "*mitra*" = "mitre"; "*nodulus*" = "small knob"; "*spora*" = "spore", referred to the nodulose, mitriform spores

Macrocharacters—Pileus subglobose or ellipsoid-paraboloid when still closed, up to 2 mm high, convex-conic to conic-campanulate later, expanding to convex-plane or even revolute with an even margin, not umbonate, 3–10 mm diam., wholly and densely pruinose-pubescent, pruina thinning away with age, radially striate, becoming slightly grooved. Cuticle ochreous at first, with orange to purplish, rarely olive, shades, becoming cream coloured with a darker disc, finally greyish; lamellae ascendant, free, ventricose, thin, distant, black at maturity with a paler edge. lamellulae present; stipe up to 45 × 0.5–0.8 mm, whitish or slightly paler than cap, wavy, cylindric, somewhat enlarged but not bulbous at the base, hollow, entirely pruinose-pubescent, often with a radial, white mycelial felt; veil granulose, present both on the cap and stem; context imperceptible. No smell.

Microcharacters—Basidiospores (9–)9.5–11(–11.5) × 6–7 × 5–6 µm, mitriform in frontal view (Q = 1.38–1.69; Q average = 1.52), subamygdaliform in side view, with a conical base and conical or convex apex, nodulose usually having two knobs on each side in face view, dark reddish brown at maturity, with a well developed, prominent apiculus, and an eccentric germ pore, 1.5–2 µm diam.; basidia 4-spored, 16–27 × 6–9 µm, bimorphic, claviform or subcylindric, the latter with a slight median constriction, each surrounded by 4–5 globose to claviform brachybasidia, 17–33 × 17–30 µm.; pleurocystidia absent; cheilocystidia abundant, globose or broadly ellipsoidal, with a pedicel, 25–39 × 21–32 µm.; pileipellis a hymeniderm of globose, claviform, or broadly ellipsoidal, sometimes encrusted cells, 20–48 ×16–34 µm.; pileocystidia numerous, of two kinds, both lageniform: 1) thin-walled (leptocystidia), 62–78(–90) × 12–15µm, bulbous at the base, with a neck tapering upwards,

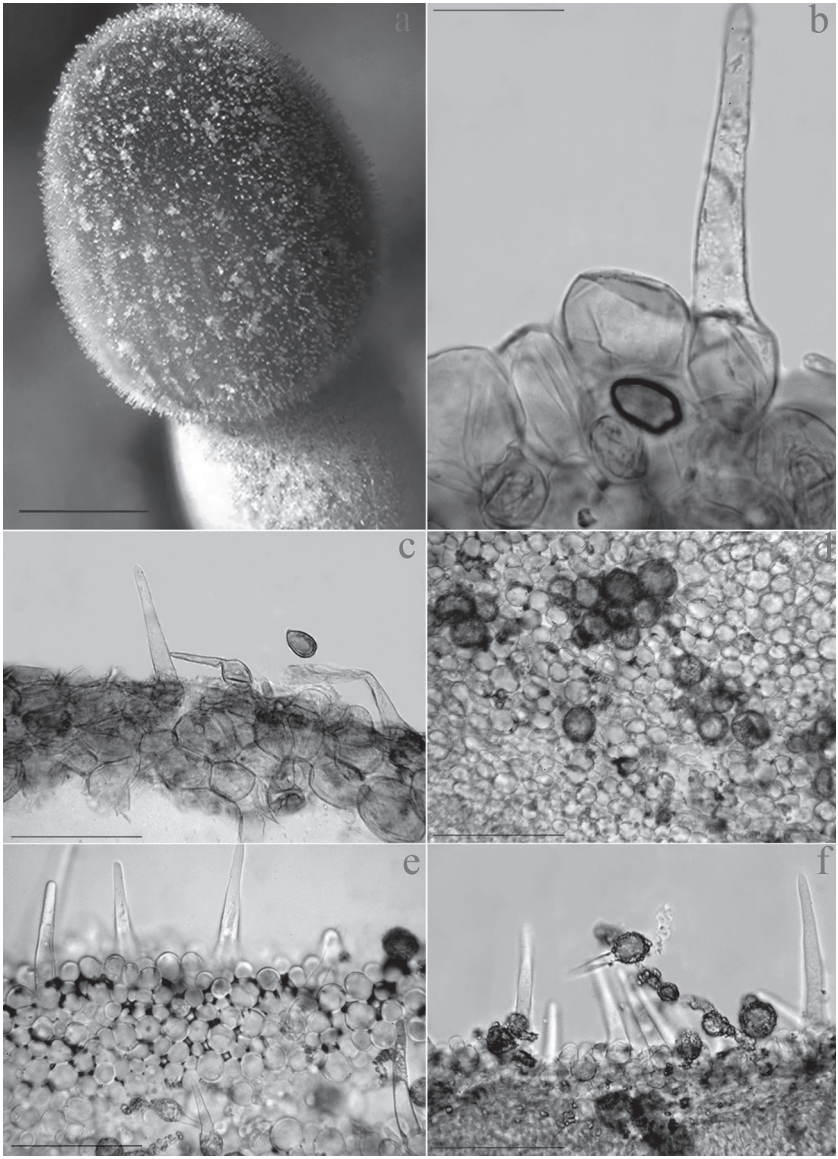

Fig. 1 *Coprinellus mitrinodulisporus* (holotype): a = basidioma in an early stage; b = hymenidermal cells interspaced with a leptopileocystidium; c, e–f = details of pileipellis with leptocystidia, sclerocystidia, and dark pigmented veil cells; d = dark pigmented veil cells above the hymenidermal cells. Scale bars: a = 500 µm; b = 20 µm; c–f = 50 µm.

7–10 µm diam. at their base, sometimes sparsely encrusted at the neck and densely and coarsely at the base; 2) thick-walled (sclerocystidia), 35–45 × 11–16 µm (3–4 µm diam. at the neck base), darker than leptocystidia, usually coarsely encrusted at their bulbous base; CAULOCUTIS with the outermost hyphae 1–3 µm diam., sometimes encrusted, supporting many cystidia similar to pileocystidia, 40–75 × 10–17 µm.; VEIL formed of coarsely encrusted, thick-walled sphaerocysts, 12–20 µm diam., globose or even in transitional forms, with hints of neck, from sphaerocysts to sclerocystidia; CLAMP-CONNECTIONS absent.

ECOLOGY, RANGE, DISTRIBUTION—About twenty scattered specimens on chamois (*Rupicapra rupicapra*) dung in a damp chamber culture. August. To date only known from the type locality.

MOLECULAR ATTRIBUTES—Amplification of the LSU and ITS regions resulted in about 1.4 kb and 600 bp long sequences, respectively. Comparison of our LSU sequence (accession number HQ180170) with those deposited in GenBank resulted in high similarity percentages (96%) with other strains of *Coprinellus* spp., and comparison of the ITS sequence (accession number HQ180171) within the same database confirmed this result. A β-tubulin sequence has been deposited (HQ180172) to support further phylogenetic studies on *C. mitrinodulisporus*.

Discussion

The main features of *Coprinellus mitrinodulisporus* are growth on dung, pileus with setulae, and a granulose, sphaerocystic veil, the latter particularly evident in the early stages, mitriform and nodulose basidiospores, and absence of clamp-connections. The presence of a hymenidermal pileipellis and setuliform pileo- and caulocystidia places the species in subs. *Setulosi* of Uljé & Bas (1991) and now in *Coprinellus*, as revised and reinstated by phylogenetic studies (Redhead et al. 2001) as section *Setulosi* (J.E. Lange) D.J. Schaf. (Schafer 2010).

Coprinellus mitrinodulisporus is very close to *Coprinus doverii* L. Nagy, a typical representative of *Setulosi* not yet recombined in *Coprinellus* (Nagy, in litt.). The two species share habitat and many macro- and microscopic features, including encrusted lageniform pileocystidia and mitriform nodulose spores, but *C. mitrinodulisporus* differs in having larger spores (6.2–8.3 × 4.5–5.8 × 3.8–4.1 µm in *C. doverii*), abundant and larger cheilocystidia (gill edge almost sterile), longer pileocystidia, abundant sclerocystidia and veil (the latter easily observable with a ×10 magnification), and in lacking clamp connections, which are absent also in the mycelial felt. In addition, *C. mitrinodulisporus* has pileocystidia with constantly tapering necks rather than with both tapering and cylindrical necks. Given the limited number of collections of both species

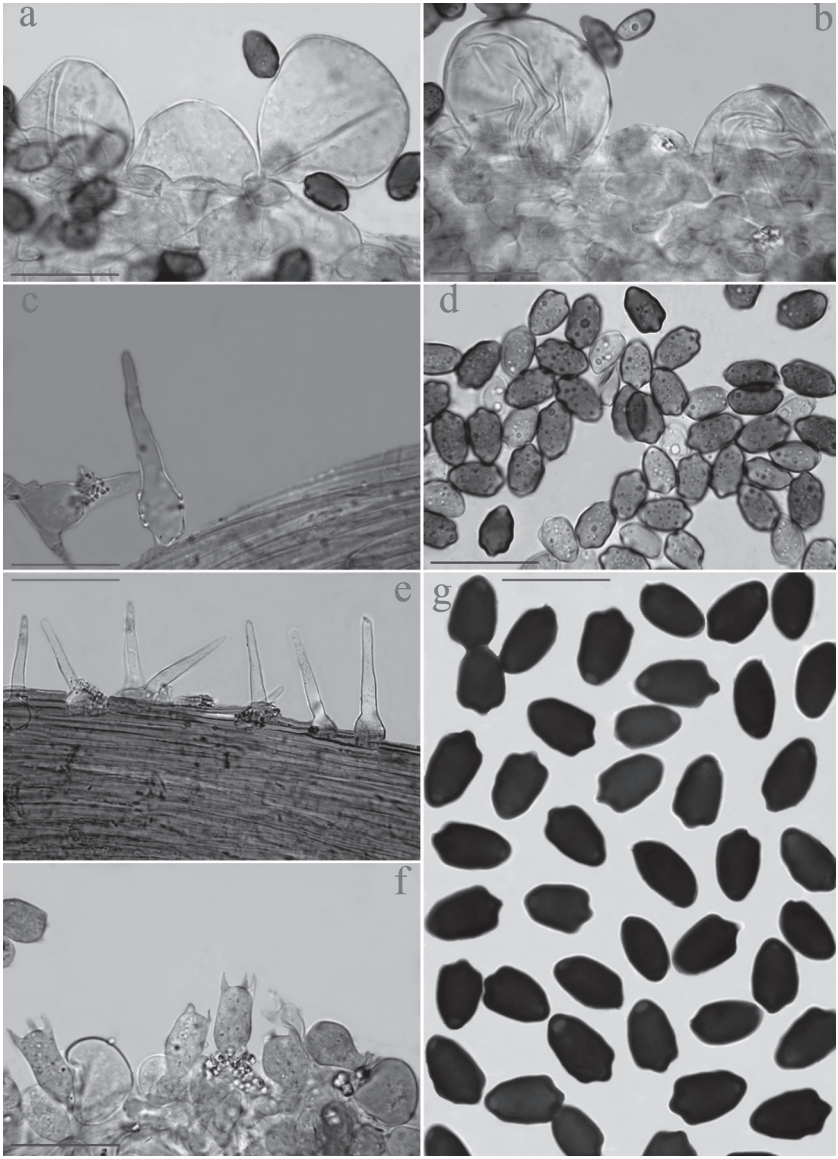

Fig. 2 *Coprinellus mitrinodulisporus* (holotype): a = brachybasidia; b = cheilocystidia; c,e = details of caulocutis with lageniform cystidia; d = immature and maturing spores; f = detail of hymenium with basidia; g = mature spores. Scale bars: a–d, f = 20 µm; e = 50 µm; g = 15 µm.

studied and the unique combination of characters they share, they might conceivably represent one, variable taxon. Further studies are clearly desirable but considering the observed differences and the possible rarity of the taxa, we prefer to treat these as separate species. As the two species occupy an isolated morphological position in *Setulosi*, a molecular phylogeny might not clearly differentiate them from each other. It would be interesting to explore their intersterility in mating studies.

Lageniform leptopileo- and scleropileocystidia tapering upwards and similarly sized (8.5–11 × 6.5–8.5 × 5–6.5 µm in Orton & Watling 1979), mitriform basidiospores are also found in *Coprinellus angulatus* (Peck) Redhead et al., which is, however, a carbonicolous species with much larger, rust-brown fruitbodies, non-nodulose, more squat basidiospores (average length/breadth = 1.25–1.35, Uljé 2005) with a central, very wide and truncate germ pore, pleurocystidia, and clamp-connections.

Coprinellus marculentus (Britzelm.) Redhead et al., a coprophilous pileocystidiate species with a granular veil and similarly sized basidiospores also shares purplish pileus shades and globose or broadly ellipsoidal cheilocystidia (Uljé & Bas 1991), but *C. marculentus* differs in its smooth, usually hexagonal, sometimes mitriform basidiospores, and pileocystidia with a cylindrical neck, equal or enlarged at its apex. It also differs from *C. mitrinodulisporus* in lacking sclerocystidia and having pleurocystidia and clamp-connections.

Although it does not have mitriform spores, *Coprinellus heptemerus* (M. Lange & A.H. Smith) Vilgalys et al. has other characters in common with *C. mitrinodulisporus,* including an encrusted veil with cells transitional between sphaerocysts and pileocystidia, a lack of clamp connections and pleurocystidia, small fruitbodies, and a habit on dung. However, the combination of characters and distinctly shaped spores distinguish *C. mitrinodulisporus* clearly from *C. heptemerus* and other previously published *Setulosi,* except *C. doverii*.

Apart from *C. doverii*, no other *Setulosi* species published after Uljé & Bas (1991) and Uljé & Noordeloos (2003) has coarsely encrusted veil sphaerocysts, sclerocystidia and mitriform basidiospores, easily distinguishing them from *C. mitrinodulisporus*. We take the opportunity to recombine some of them in *Coprinellus*:

Coprinellus allovelus (Uljé) Doveri & Sarrocco, **comb.nov.**

MycoBank 518736

≡ *Coprinus allovelus* Uljé, in Uljé & Noordeloos, Persoonia 18: 261, 2003

Coprinellus limicola (Uljé) Doveri & Sarrocco, **stat. nov.**, **comb.nov.**

MycoBank 518737

≡ *Coprinus callinus* var. *limicola* Uljé, in Uljé & Noordeloos, Persoonia 18: 259, 2003 as "*limicolus*"

Note: Nagy (pers. comm.) reports that, based on molecular results, this is a separate species. Morphologically it has a number of differences from *C. callinus* that support its rank as a distinct species. M. Lange (1952), who reported that collections identified morphologically as *C. callinus* consisted of two intersterile taxa, was not able to distinguish these morphologically.

Coprinellus canistri (Uljé & Verbeken) Doveri & Sarrocco, **comb.nov.**

MycoBank 518738

≡ *Coprinus canistri* Uljé & Verbeken, Persoonia 18: 143, 2002

Coprinellus minutisporus (Uljé) Doveri & Sarrocco, **comb.nov.**

MycoBank 518739

≡ *Coprinus minutisporus* Uljé in Uljé & Noordeloos, Persoonia 18: 260, 2003

Coprinellus pseudoamphithallus (Uljé) Doveri & Sarrocco, **comb.nov.**

MycoBank 518741

≡ *Coprinus pseudoamphithallus* Uljé in Uljé & Noordeloos, Persoonia 18: 263, 2003

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